

CLAIMS

WHAT IS CLAIMED IS:

- 5 1. A nucleic acid molecule comprising a nucleic acid sequence having at least 15 contiguous nucleotide length complementary to a nucleic acid sequence encoding a prolamin polypeptide, or a nucleic acid sequence having at least about 70 % homologous to the complementary nucleic acid sequence
10 having at least 15 contiguous nucleotide length.
2. The nucleic acid molecule according to Claim 1 comprising said nucleic acid sequence having at least 15 contiguous nucleotide length complementary to a nucleic acid
15 sequence encoding a prolamin polypeptide.
3. The nucleic acid molecule according to Claim 1, wherein the prolamin is of rice.
- 20 4. The nucleic acid molecule according to Claim 1, wherein the prolamin is of japonica rice.
5. The nucleic acid molecule according to Claim 1, wherein the complementary nucleic acid sequence having at least 15
25 contiguous nucleotide length has at least 50 contiguous nucleotide length.
6. The nucleic acid molecule according to Claim 1, wherein the complementary nucleic acid sequence having at least 15 contiguous nucleotide length comprises a full length sequence
30 encoding the prolamin polypeptide.
7. The nucleic acid molecule according to Claim 1, wherein the complementary nucleic acid sequence having at least 15

contiguous nucleotide length is a 5' terminal nucleic acid sequence encoding the prolamin polypeptide.

8. The nucleic acid molecule according to Claim 1, wherein
5 the at least 15 contiguous nucleotide length complementary,
is a nucleotide length of 50 nucleotide or less.

9. The nucleic acid molecule according to Claim 1, wherein
the at least 15 contiguous nucleotide length complementary,
10 is a nucleotide length of 30 nucleotide or less.

10. The nucleic acid molecule according to Claim 1, wherein
the at least 15 contiguous nucleotide length complementary,
comprises a sequence having at least 15 nucleotide length
15 of a nucleic acid sequence encoding an amino acid sequence
selected from the group consisting of SEQ ID NOs; 98-101.

11. The nucleic acid molecule according to Claim 1, wherein
the prolamin is a 13 kDa prolamin.
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12. The nucleic acid molecule according to Claim 1,
comprising a nucleic acid sequence of at least 15 contiguous
nucleotide length, complementary to:

(a) a polynucleotide having a nucleic acid sequence set forth
25 in a SEQ ID NO, selected from the group consisting of SEQ
ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27,
29, 31, 33, 35, 37, 39, 41, 43 and 45, or a fragment sequence
thereof;

(b) a polynucleotide encoding a polypeptide having an amino
30 acid sequence set forth in SEQ ID NO: selected from the group
consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18,
20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 46,
or a fragment sequence thereof;

(c) a polynucleotide encoding a polypeptide variant having at least one mutation selected from the group consisting of one or more amino acid substitution, addition and deletion in an amino acid sequence set forth in SEQ ID NO: selected
5 from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 46, and having a biological activity;

(d) a polynucleotide of an allelic variant of a DNA consisting of a nucleic acid sequence set forth in a SEQ ID NO, selected
10 from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 and 45;

(e) a polynucleotide encoding a species homolog or an ortholog of a polypeptide consisting of an amino acid sequence set
15 forth in SEQ ID NO: selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 46;

(f) a polynucleotide hybridizing to at least one polynucleotide of any of (a)-(e), and encoding a polypeptide
20 having a biological activity; or

(g) a polynucleotide consisting of a base sequence having at least 70 % identity with at least one polynucleotide of (a)-(e) or a complementary sequence thereof, and encoding a polypeptide having a biological activity.
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13. The nucleic acid molecule according to Claim 1, having antisense activity.

14. The nucleic acid molecule according to Claim 13,
30 wherein the antisense activity reduces expression of the prolamin polypeptide.

15. A nucleic acid molecule comprising a nucleic acid

sequence having at least 15 contiguous nucleotide length
of a nucleic acid sequence encoding a prolamin polypeptide,
or a nucleic acid sequence having at least about 70 % homologous
to the nucleic acid sequence having at least 15 contiguous
5 nucleotide length.

16. A nucleic acid molecule comprising:

A nucleic acid sequence A comprising a nucleic acid sequence
having at least 15 contiguous nucleotide length of a nucleic
acid sequence encoding a prolamin polypeptide, or a nucleic
10 acid sequence having at least about 70 % homologous to the
nucleic acid sequence having at least 15 contiguous
nucleotide length; and

(B) a nucleic acid sequence B comprising a nucleic acid
sequence having at least 15 contiguous nucleotide length
15 complementary to a nucleic acid sequence encoding a prolamin
polypeptide, or a nucleic acid sequence having at least about
70 % homologous to the complementary nucleic acid sequence
having at least 15 contiguous nucleotide length

20 17. The nucleic acid molecule according to Claim 16, wherein
the nucleic acid sequence A and the nucleic acid sequence
B have a portion substantially complementary to each other.

18. The nucleic acid molecule according to Claim 16,
25 wherein the nucleic acid sequence A and the nucleic acid
sequence B are substantially complementary to each other.

19. The nucleic acid molecule according to Claim 16,
further comprising a spacer sequence.

30 20. The nucleic acid molecule according to Claim 19,
wherein the spacer sequence comprises an intron sequence.

21. The nucleic acid molecule according to Claim 19, wherein the space sequence is comprised between the nucleic acid sequence A and the nucleic acid sequence B.

5 22. An agent causing RNA interference (RNAi) against a gene sequence encoding a prolamin polypeptide.

23. A nucleic acid cassette comprising a nucleic acid sequence B comprising a nucleic acid sequence having at least
10 15 contiguous nucleotide length complementary to a nucleic acid sequence encoding a prolamin polypeptide, or a nucleic acid sequence having at least about 70 % homologous to the complementary nucleic acid sequence having at least 15 contiguous nucleotide length.

15 24. The nucleic acid cassette according to Claim 23, further comprising a nucleic acid sequence encoding a foreign gene.

20 25. The nucleic acid cassette according to Claim 23, further comprising a nucleic acid sequence A comprising a nucleic acid sequence having at least 15 contiguous nucleotide length of a nucleic acid sequence encoding a prolamin polypeptide, or a nucleic acid sequence having at
25 least about 70 % homologous to the nucleic acid sequence having at least 15 contiguous nucleotide length.

26. The nucleic acid cassette according to Claim 25, further comprising a spacer sequence.

30 27. The nucleic acid cassette according to Claim 26, wherein the space sequence comprises an intron sequence.

28. The nucleic acid cassette according to Claim 26, wherein the spacer sequence is comprised between the nucleic acid sequence A and the nucleic acid sequence B.

5 29. The nucleic acid cassette according to Claim 25, further comprising a signal cassette.

30. The nucleic acid cassette according to Claim 29, wherein the signal sequence is located upstream of the foreign
10 gene.

31. The nucleic acid cassette according to Claim 29, wherein the signal sequence is a signal sequence of a storage
15 protein.

32. The nucleic acid sequence according to Claim 29, wherein the signal sequence is a prolamin signal sequence.

33. The nucleic acid cassette according to Claim 24,
20 further comprising a promoter sequence.

34. The nucleic acid cassette according to Claim 33, wherein the promoter sequence is operably linked to both the foreign gene and the nucleic acid sequence B.
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35. The nucleic acid cassette according to Claim 24, wherein separate promoters are independently operably linked to the foreign gene and the nucleic acid B.

30 36. The nucleic acid cassette according to Claim 35, wherein a promoter sequence operably linked to the foreign gene (promoter sequence A), and a promoter sequence operably linked to the nucleic acid sequence B (promoter sequence

B), are different to each other.

37. The nucleic acid cassette according to Claim 36,
wherein the promoter sequence B is a promoter promoting
5 expression in a high level in seeds.

38. The nucleic acid cassette according to Claim 36,
wherein the promoter sequence B is derived from a storage
protein promoter.
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39. The nucleic acid cassette according to Claim 36,
wherein the promoter sequence B is derived from a storage
protein promoter, and is different from the promoter sequence
A.
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40. The nucleic acid cassette according to Claim 36 wherein
the promoter sequence B is derived from a promoter selected
from the group consisting of a polyubiquitin promoter, 26
kD globulin promoter, glutelin A promoter, glutelin B
20 promoter, 16kD prolamin promoter, 13kD prolamin promoter
and 10 kD prolamin promoter.

41. The nucleic acid cassette according to Claim 36 wherein
the promoter sequence A is derived from a storage protein
25 promoter.

42. The nucleic acid cassette according to Claim 36,
wherein the promoter sequence A is a promoter sequence
naturally associated with the nucleic acid sequence B.
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43. The nucleic acid cassette according to claim 36 wherein
the promoter sequence A is derived from a promoter selected
from the group consisting of 26 kD globulin promoter, glutelin

A promoter, glutelin B promoter, 16kD prolamin promoter, 13kD prolamin promoter and 10 kD prolamin promoter.

44. The nucleic acid cassette according to Claim 36,
5 wherein the promoter sequence A is a prolamin promoter.

45. The nucleic acid cassette according to Claim 36,
wherein the promoter sequence A is derived from a prolamin
promoter, and the promoter sequence B is derived from a
10 promoter other than the prolamin promoter.

46. The nucleic acid cassette according to Claim 33,
comprising a signal sequence between the foreign gene and
the promoter sequence in frame.

15 47. The nucleic acid cassette according to Claim 25 further
comprising a terminator sequence.

48. The nucleic acid cassette according to Claim 47,
20 wherein the terminator sequence is a terminator sequence
of 10 kD prolamin.

49. The nucleic acid cassette according to Claim 25,
further comprising a foreign gene, and the foreign gene is
25 located upstream of both the nucleic acid sequence A and
the nucleic acid sequence B.

50. The nucleic acid cassette according to Claim 49
comprising a spacer sequence between the nucleic acid
30 sequence A and the nucleic acid sequence B.

51. The nucleic acid cassette according to Claim 49
comprising an intron sequence between the nucleic acid

sequence A and the nucleic acid sequence B.

52. A method for producing a nucleic acid cassette comprising the steps of:

- 5 A) providing a nucleic acid cassette comprising a set of a nucleic acid cassette comprising a nucleic acid sequence B comprising a nucleic acid sequence having at least 15 contiguous nucleotide length complementary to a nucleic acid sequence encoding a prolamin polypeptide, or a nucleic acid sequence having at least about 70 % homologous to the complementary nucleic acid sequence having at least 15 contiguous nucleotide length, and a nucleic acid sequence A comprising a nucleic acid sequence having at least 15 contiguous nucleotide length of a nucleic acid sequence encoding a prolamin polypeptide, or a nucleic acid sequence having at least about 70 % homologous to the nucleic acid sequence having at least 15 contiguous nucleotide length, a promoter sequence B upstream of the set, a foreign gene located upstream or downstream of the promoter sequence B, and a promoter sequence A operably linked to the foreign gene;
- 10 B) transforming a plant with the nucleic acid cassette; and
- 15 C) selecting a nucleic acid cassette having the expression amount of prolamin is partially reduced with respect to the transformed plant.
- 20 D) selecting a nucleic acid cassette having the expression amount of prolamin is partially reduced with respect to the transformed plant.
- 25 E) selecting a nucleic acid cassette having the expression amount of prolamin is partially reduced with respect to the transformed plant.

53. A vector comprising the nucleic acid molecule according to Claim 1.

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54. The vector according to Claim 53, further comprising a sequence having a promoter activity.

55. The vector according to Claim 54, wherein the sequence having the promoter activity is a storage protein promoter.

56. The vector according to Claim 53 wherein the sequence
5 having the promoter activity is a promoter of the prolamin.

57. The vector according to Claim 53, further comprising a terminator.

10 58. The vector according to Claim 53, further comprising a sequence encoding a selectable marker.

59. The vector according to Claim 53, further comprising a sequence encoding a foreign gene different from the nucleic
15 acid molecule according to Claim 1.

60. A plant cell comprising the nucleic acid molecule according to Claim 1.

20 61. The plant cell according to Claim 60, further comprising a nucleic acid molecule encoding a foreign gene different from the nucleic acid molecule according to Claim 1.

25 62. The plant cell according to Claim 60, wherein the plant species from which the prolamin is derived, and the species of the plant are of the same species.

30 63. The plant cell according to Claim 60 wherein the plant species from which the prolamin is derived, and the species of the plant are of the same variant.

64. The plant cell according to Claim 60, wherein the

species from which the prolamin is derived and the species of the plant are of rice.

5 65. The plant cell according to Claim 60, wherein the species from which the prolamin is derived and the species of the plant are of a *japonica* rice.

10 66. The plant cell according to Claim 60, having the nucleic acid molecule of Claim 1 introduced in both alleles thereof.

67. A plant tissue comprising the plant cell according to Claim 60.

15 68. A plant body comprising the nucleic acid molecule of Claim 1.

20 69. The plant body according to Claim 68, further comprising a nucleic acid molecule encoding a foreign gene different from the nucleic acid molecule according to Claim 1.

70. The plant body according to Claim 68, which is of the same species as that from which the prolamin is derived.

25 71. The plant body according to Claim 68, which is of the same variant as that from which the prolamin is derived.

30 72. The plant body according to Claim 68, wherein the plant species from which the prolamin and the plant body are derived, are rice.

72. The plant body according to Claim 68, wherein the plant species from which the prolamin and the plant body are derived,

are *japonica* rice.

74. The plant body according to Claim 68, having the nucleic acid molecule of Claim 1 introduced in both alleles thereof.

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75. A plant seed produced from the plant body according to Claim 68.

76. A plant seed produced from the plant body according to Claim 69.

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77. A starch preparation produced from the plant body according to Claim 68, or the plant seed according to Claim 75.

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78. A composition comprising a gene product of the foreign gene produced from the plant body according to Claim 69 or the plant seed according to Claim 76.

79. A method for reducing an expression amount of a protein in a seed in a plant, comprising the steps of:

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A) providing the nucleic acid molecule according to Claim 1;

B) introducing the nucleic acid molecule into the plant;

25 C) redifferentiating the cell to produce a transgenic plant; and

D) obtaining a seed from the transgenic plant.

80. The method according to Claim 79, wherein the step of introducing is performed by *Agrobacterium* method.

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81. The method according to Claim 79, further comprising the step of E) selecting a plant cell with the nucleic acid

introduced therein.

82. The method according to Claim 81, wherein the step
of selecting is performed by determining resistance against
5 an antibiotic.

83. A method for expressing a foreign gene in a plant seed,
comprising the steps of:
providing the nucleic acid molecule according to Claim 1;
10 providing a nucleic acid encoding the foreign gene;
introducing the nucleic acid molecule according to Claim
1 and the nucleic acid encoding the foreign gene, into a
cell of the plant;
redifferentiating the cell to produce a transgenic plant;
15 and
obtaining a seed from the transgenic plant.

84. The method according to Claim 83, wherein the step
of introducing is performed by *Agrobacterium* method.
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85. The method according to Claim 83, further comprising
the step of F) selecting a plant cell with the nucleic acid
molecule introduced.

25 86. The method according to Claim 85, wherein the step
of selecting is carried out by determining resistance of
the plant cell against an antibiotic.

87. The method according to Claim 83, further comprising
30 the step of G) separating a gene product of the foreign gene
from the seed.

88. A composition comprising a gene product of the foreign

gene produced by the method according to Claim 83.

89. Use of a nucleic acid molecule according to Claim 1
for reducing expression amount of a protein in a seed of
5 a plant.

90. Use of a nucleic acid molecule according to Claim 1
for expressing a foreign gene in a seed of a plant.

10 91. Use according to Claim 90, wherein the expression of
native proteins of the plant in the seed is reduced.